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HIV-1 Tat Protein Promotes Neuroendocrine Dysfunction Concurrent with the Potentiation of Oxycodone's Psychomotor Behavioral Effects in Female Transgenic Mice

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Abstract

Human immunodeficiency virus (HIV) is associated with neuroendocrine dysfunction which may contribute to co-morbid stress-sensitive disorders. The hypothalamic-pituitary-adrenal (HPA) and -gonadal (HPG) axes are perturbed in up to 50% of HIV patients, but the mechanisms are not known, but we find that transgenic expression of Tat protein to recapitulate the clinical phenotype in male mice. We hypothesized that HPA and/or HPG dysregulation contributes to Tat-mediated interactions with oxycodone, a clinically-used opioid often prescribed to HIV patients. Mice that expressed the Tat_{1-86} protein [Tat(+) mice] or their control counterparts that did not [Tat(-) control mice] were exposed to forced swim stress (or not) and behaviorally-assessed for motor behavior in the open field task. Mice were pretreated with vehicle, RU-486, or antalarmin to block glucocorticoid receptors (GR) or CRF receptors (CRF-R), respectively and some mice were ovariectomized. Circulating corticosterone was assessed via enzyme-linked immunosorbant assay. In transgenic female mice, HIV Tat elevated corticosterone levels recapitulating the clinical HIV phenotype. We have found males to additionally express adrenal insufficiency in response to Tat exposure, however, this was not observed in females. When challenged with oxycodone, Tat-exposed females demonstrated a potentiated psychomotor response that was not attenuated with via GR or CRF-R inhibition. However, ovariectomy elevated hypothalamic allopregnanolone levels concurrent with significant attenuation of the psychomotor response revealing gonadal hormones for Tat-mediated potentiation of oxycodone. In the non-stressed or stressed paradigm, either oxycodone-administered mice or diestrous mice demonstrated a significant increase in hypothalamic allopregnanolone indicative of central adaptive response to stress. Together, these effects support the notion that Tat exposure dysregulates the HPA and HPG axes, potentially raising vulnerability to stress-related substance use disorders in females. HPG activation may be necessary for the combined oxycodone-Tat interaction indicating the importance of maintained HPG feedback in this vulnerable population.

Hypotheses

•***In vivo,* HIV-1 Tat and oxycodone will interact to potentiate psychomotor behavior involving hypothalamic-pituitary-adrenal (HPA) axis activation.**

•HPA blockade by (Antalarmin or RU-486) or HPG blockade by OVX may attenuate combined Tat and oxycodone psychomotor behavior and restore HPA response.

•Exposure to Tat and/or oxycodone may increase central Allopregnanolone levels.

Methods

Animal Subjects: Transgenic mice were bred in the vivarium at the University of Mississippi (University, MS). Tat(+) mice expressed a Tat $_{1-86}$ protein that became transcriptionally-active in the presence of doxycycline (induced via doxycycline injection, 30mg/kg/d for 5d). Tat(−) mice expressed the transcription factor necessary to activate transgene induction, but did not express the transgene itself. Psychomotor and anxiogenic effects of Tat induction have been previously-observed using these mice [1-3]. Mice were kept in a temperature- and humidity-controlled environment on a 12:12 h light:dark cycle (lights off at 09:00 h) with *ad libitum* access to food and water.

Estrous Cycle phase & Behavioral Assessment: Estrous cycle was determined via vaginal lavage conducted daily. Mice were behaviorally-tested in proestrous and diestrous cycle phase followed by swim stress challenge (stressed paradigm) or not (non-stressed paradigm) and then assessed in an open field task. All tests were completed within 14 days of doxycycline induction and occurred 2-3 h into the dark phase of the light cycle. Data were encoded by an ANY-maze behavioral tracking system (Stoelting Co., Wood Dale, IL). Some mice were ovariectomized and allowed for 1 week for hormone washout followed by Tat induction and behavior assessment.

Chemicals: Tat_{1-86} was induced in transgenic mice [Tat(+) or Tat(−)] via doxycycline injection (30 mg/kg, i.p.; Cayman Chemical, Ann Arbor, MI). Antalarmin (20 mg/kg, i.p.; 7 days Cayman Chemical, Ann Arbor, MI) and/or RU-486 (20 mg/kg, i.p.; 8 days Cayman Chemical, Ann Arbor, MI). Oxycodone (3 mg/kg, i.p.; Sigma-Aldrich, MO, USA) or saline (0.9%) was administered once 15 min prior to testing.

Enzyme-linked immunosorbent assay (ELISA): Circulating corticosterone was assessed via ELISA kit per manufacturer instructions (Neogen Life Sciences). Plates were read on a CLARIOstar microplate reader (BMG Labtech Inc., Cary, NC).

Ultra Performance Liquid Chromatography (UPLC) - Mass Spectrometry (MS): Central Allopregnanolone levels were assessed via UPLC-MS per the standard operating procedure.

HIV-1 Tat promoted HPA dysregulation and potentiated Oxycodone-mediated psychomotor behavior

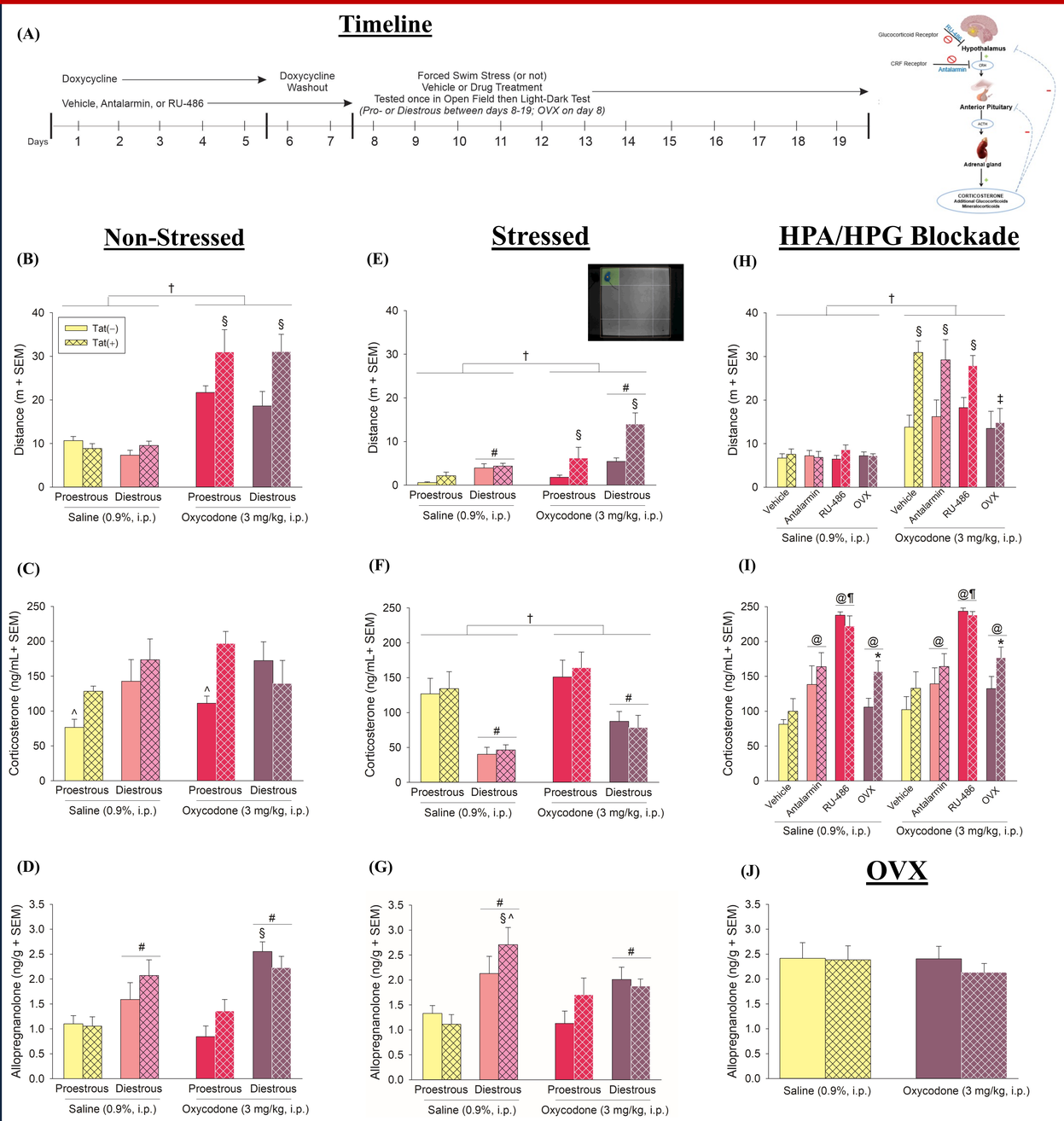

Figure 1: Tat_{1-86} was induced in Tat(+) and not induced in Tat(−) mice via doxycycline injection (30mg/kg for 5 d) . Saline or oxycodone (3mg/kg) were administered 15 minutes prior to behavior testing and assessed in an open field (n = 8-10); **(A) Behavior Timeline. Distance (m)** traveled in open field after (n=8-10), **Circulating corticosterone** (ng/mL; n=7-10) and **hypothalamic allopregnanolone** (n=8 tissues/group) were assessed in **Non-stressed** (Acute administration of saline or oxycodone in panels B,C,D); **Stressed** (Exposure to forced swim stress stimuli followed by acute administration of saline or oxycodone in panels **E,F,G**) and HPA/HPG Blockade (Administration of Vehicle, Antalarmin, RU-486 or OVX followed by administration of saline and oxycodone in panels **H & I)** and OVX (in panel **J**). † indicates a main effect for oxycodone-administered mice to differ from saline administered controls **in panels B, E, F, H**. § indicates an interaction wherein oxycodone-administered Tat(+) mice differ from respective Tat(−) controls and saline-administered controls **in panels B, E, H**, ‡ indicates an interaction wherein oxycodone-administered Tat(+) OVX mice differ from all other oxycodone- administered Tat(+) groups **in panel H**, ^ indicates an interaction wherein the denoted group differs from all other groups **in panel C, #** indicates a main effect for proestrous mice to differ from diestrous mice **in panel D, E, F, G.** @ indicates an interaction wherein the denoted group differs from their respective vehicle controls in panel I, ¶ indicates an interaction wherein mice pretreated with RU-486 differ from all other groups in panel I, § indicates an interaction wherein denoted group differs from Tat(−) or Tat(+) proestrous mice **in panel D & G**. ^ indicates an interaction wherein the denoted group differs from oxycodone-administered, Tat(+) diestrous mice **in panel G**, $p < 0.05$.

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Summary

- Tat expression interacted with oxycodone to potentiate psychostimulatory effects (**Fig. 1B**).
- When stressed, mice in diestrous phase traveled significantly greater distance than their proestrous counterparts (**Fig. 1E**).
- OVX significantly attenuated combined Tat and oxycodone mediated psychomotor behavior (**Fig. 1H**)
- Tat expression and/or oxycodone exposure increased circulating corticosterone at baseline compared to proestrous Tat(−) controls, recapitulating the clinical phenotype (**Fig. 1C**).
- When stressed, proestrous and/or oxycodone exposure significantly produced higher circulating corticosterone levels (**Fig. 1F**)
- Unlike males, adrenal insufficiency was not observed in female mice, demonstrated by a increase in circulating corticosterone levels in Antalarmin, RU-486 or OVX group when compared to vehicle controls (**Fig. 1I**).
- A significant increase in hypothalamic allopregnanolone was observed when mice were in diestrous phase and/or exposed to oxycodone. (**Fig. 1 D,G**)

Conclusions

HIV Tat and clinical opioids can interact to activate stress pathways, influencing psychomotor behavior which may involve HPA axis dysregulation. OVX and not Antalarmin or RU-486 attenuated the psychomotor behavior implicating gonadal hormones as drivers for neuroHIV behavior in female mice. Thus, neuroendocrine axis may be an important target for combined HIV-1 Tat and opioid interactions within the HIV infected population.

References

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